The Pharma Innovation Journal 2023; 12(3): 1493-1494

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 1493-1494

www.thepharmajournal.com
Received: 21-12-2022
Accepted: 24-01-2023

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Vaginal cytology: Method for detection of estrus in canine

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Abstract
Vaginal cytology has many practical applications in the evaluation of both the normal and abnormal bitch. The objective of this article is to describe the use of exfoliative vaginal cytology as a diagnostic tool in clinical canine reproduction. Vaginal cell were collected with the help of sterile AI sheath and make thin smear. Fixed with methanol after giemsa stainig see under light microscope and evaluate of vaginal smear at 400X. Under microscope four type of cells that is Parabasal, Intermediate, Superficial and Anuclear were observed in different stage of estrus cycle.

Keywords: Vaginal cytology, canine, bitch, AI sheath, normal saline, parabasal, intermediate, superficial, anuclear

Introduction
Vaginal cytology is a rapid, inexpensive, and reliable method to evaluate stages of the estrus cycle in the bitch. There is 4 phases of canine estrous cycle: proestrus, estrus, diestrus and anestrous. Proestrus and estrus are commonly called "heat" or "season". Finding the day of ovulation during estrus is essential for an appropriate timing of mating or insemination and, thus, a high fertility (Romagnoli, 2017) [11] A blood tinged vaginal discharge is present and the vulva is moderately enlarged and turgid. it can be helpful to determine other stages of the estrus cycle, i.e. proestrus or diestrus. Also abnormal estrus cycle patterns, such as early or late ovulation, silent heats or split heats can be diagnosed (Goodman, 2001) [6]. The cells from vaginal cytology smears change over a period of 4 to 7 days from parabasal cells, intermediate cells, superficial cells, and anuclear cells. Parabasal and intermediate cells are the non-confirmed cells where as superficial and anuclear cells are confirmed cells. These changes in the vaginal cytology reflect the increasing estrogen from the ovarian follicles. Red blood cells are usually, but not invariably, present. Proestrus can last from 3 days to 3 weeks, with 9 days being the average. Vaginal cytology is the most popular diagnostic method as a part of the gynaecological examination in the bitch (Groppetti *et al*., 2012; Wehrend *et al*., 2013) [7, 15]. It is based on the cyclic cellular changes occurring in the vaginal epithelium as a result of reproductive hormone that is estrogens (Wright & Parry, 1989) [16]. The method is simple and useful for most frequently the determination of the estrous cycle stages with respect to the optimal breeding time (Turmalay *et al*., 2011) [14].

Material and Method
Vaginal cell were collected using a sterile AI sheath, disposable syringe and normal saline (NS). Take 5 ml normal saline in disposable syringe and plugged with AI sheath. AI sheath insert in vagina and push normal saline. After proper mixing, suck out with the help of AI sheath and syringe. In this method there is less chance of contamination with vaginal floor. Take one drop of fluid over the slide and make thin smear. Fixed the slide with methanol. After 4-5 mints stained with giemsa stain and wait for 45 minuts. Washed with distilled water and make it air dry. After that see under light microscope and evaluate of vaginal smear at 400X. A minimum of 10 observation fields should be examined (Theise, 2002) [13].

Result and Discussion
There are different vaginal cell types viz parabasal, intermediate, superficial and anuclear cell. It is generally accepted that vaginal cell types in the smear are related to the stage of the estrous cycle, which makes the vaginal exfoliative cytology a valuable add-on test in reproductive clinical diagnostics.

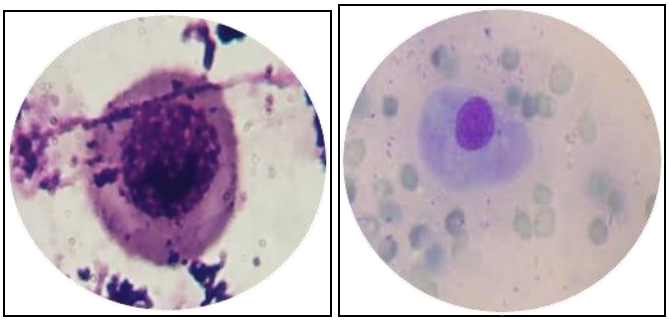
Parabasal cells are small, round with big nucleus (Fig 1A). Occasionally, they contain cytoplasmic vacuoles. Parabasal cells may also contain neutrophil granulocytes in the cytoplasm (Wright & Parry, 1989) [16].

Intermediate cells exhibit great variations in diameter, so they are differentiated as small and large type (Maneke, 2002) [10]. Both types have a well shaped nucleus. Small intermediate cells are round to elliptical, but may also have polygonal outline whereas the large type has an irregular and angulated cytoplasmic border (Christie *et al*., 1972) [4]. Intermediate cells have a prominent nucleus (Fig 1B). The large type is sometimes confused with superficial cells because they are of similar size (Johnston *et al*., 2001) [9].

Superficial cells are large cells with irregular or angulated borders and dark, pyknotic or faint nucleus (Fig 2A) (Concannon & Digregorio, 1986) [5]. Superficial cells attain their maximum at the time of estrogen peak (Johnston *et al*., 2001; Maneke, 2002; Johnson, 2006) [9, 10, 8].

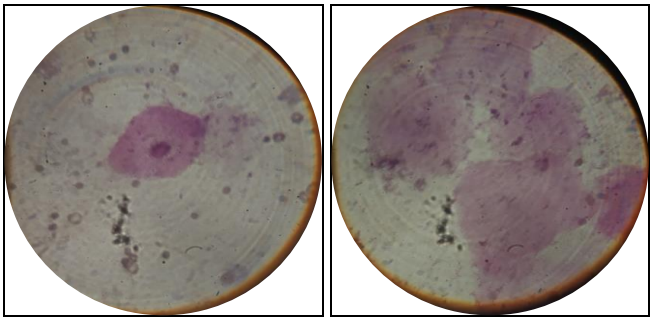
Anuclear cells are large cornified superficial cells which underwent degeneration to become dead anucleated cells (Fig 2B) (Simmons & Olson, 1989) [12]. They usually stain dark blue-purple during the estrus (Johnston *et al*., 2001) [9].

An often-used method for the determination of the cycle stage is the determination of specific percentages of cell types. Some authors claim that a typical vaginal smear in estrus has 100%; superficial cells and >80 % cells with pyknotic or absent nuclei (Bergeron *et al*., 2014) [2]. Another definition of estrus is the presence of more than 90% superficial keratinized epithelial cells (Bouchard *et al*., 1991) [3]. The onset of diestrus occurs when the number of superficial and squamous cells has decreased by at least 20% (Bouchard *et al*., 1991) [3] and when a higher number of neutrophil granulocytes are present (Aydin *et al*., 2011) [1].



A B

Fig 1: Vaginal cytology of canine shows non-confirmed cells (A Parabasal cell and B Intermediate cell)



C D

Fig 2: Vaginal cytology of canine shows confirmed cells (C Superficial cell and D Anuclear cell)

Conclusion

Vaginal cytology is a very simplicity technique because of its, accessible equipment and the possibility of getting rapid results in clinical canine reproduction. It could be used as a valuable add-on to any reproductive diagnostics in the bitch. Although it is a routine method, there are still some uninvestigated issues, for example related to the effect of copulation and artificial insemination on the dynamics of canine vaginal cell populations.

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